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SUSCEPTIBILITY OF CERTAIN DOMESTIC ANIMALS TO PLAGUE INFECTION WITH PARTICULAR REFERENCE TO THAT OF GROUND SQUIRREL ORIGIN.*

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The fact that frequently plague-infected ground squirrels have been found in pastures in California raised the old question of the susceptibility of farm animals to infection with *B. pestis*. It also seemed desirable to carry out experiments to determine the pathogenicity for domestic animals of the plague bacillus of ground squirrel origin. The work was undertaken at the suggestion of Surgeon Rupert Blue, United States Public Health and Marine Hospital Service.

The subject has been investigated by several observers with reference to the plague bacillus from man and from rats. Unfortunately some of the original communications are not now available to us.

Simpson[1] gives a review of the subject in connection with the report of his own work, and from it we glean the following data. In the tabulated results of the investigations of the German and Austrian commissions it appears that constitutional and local reactions were observed in a considerable number of cases. Disregarding intravenous and intraperitoneal inoculations, there appeared to have been no deaths proven to be due to plague among animals other than rodents and anthropoids, with the exception of some dogs and cats reported by the Austrian Commission.

We quote from Simpson's review, giving the results of experiments of certain other workers: "Haffkine experimented on horses, cows, sheep, and goats by inoculation of plague cultures, but the goats alone, without developing any acute disease, lost condition gradually, wasted away, and after a considerable time many of them succumbed. Lowson experimented on pigeons, ducks, crossbills, yellow-hammers, linnets and canaries, and failed to infect them with plague. On the other hand, Wilm, in the Hongkong epidemic of plague in 1896, succeeded in infecting a pig fed with the spleen of a man who had died of plague; and a number of poultry fed by

* Received for publication August 29, 1911.

[1] *A Treatise on Plague*, The University Press, Cambridge, 1905.

him with plague material and with pure culture of the plague bacillus died in three or four days of plague. Piaxi and Posen found that when pigeons and sparrows were starved they were susceptible to plague."

The following statements are from Simpson's account of his own work. "The result of these experiments was to establish the fact that calves, hens, turkeys, geese, pigeons, sheep, and pigs were susceptible to plague both by inoculation and by feeding, and that pigs and poultry were susceptible in a high degree.

"Of the 15 pigs experimented on 13, equal to to 86 per cent, died; of the eight calves seven, equal to 87 per cent, died; of the 31 hens 11, equal to 35 per cent, died; of the seven pigeons all died; of the six geese three, equal to 50 per cent, died; of the six turkeys four, equal to 66 per cent, died; of the six ducks all died; of the three red-beaks two died; of the seven monkeys five, equal to 70 per cent, died; of the seven guinea-pigs all died; of the 109 rats 72 per cent died."

Bannerman and Kapadia[1] analyze Simpson's observation and express themselves as not convinced from the data given that all of his animals died of plague. The following statements are condensed from their review: Lieut. Walton, I.M.S., failed to infect pigeons by hypodermic injection. London made extensive experiments in Russia with the birds of that country both in a normal state and after depression of vitality by starving, chilling, etc., and concludes that birds are not susceptible to plague. Hill, in Natal, attempted to infect domestic animals by feeding, inoculation, etc. Pigs and calves were used without result, except "transient illness due to manipulation" in the case of one of the pigs.

Bannerman and Kapadia experimented with the following animals: four pigs, four turkeys, two calves, four geese, four fowl, and four ducks. The methods of feeding, scarification, and subcutaneous inoculation were employed. One pig (scarified) was temporarily indisposed; one pig (subcutaneous inoculation) was likewise indisposed and developed a local abscess. Otherwise, the series was negative.

DeSouza, Aruda, and Pinto[2] experimented with the following animals: Two calves, eight pigs, six dogs, 10 rabbits, two ferrets, one goat, eight pigeons, one turkey, 85 chickens, and two cats. Aside from a few local and temporary reactions, and deaths not proven to be due to plague, their positive results comprise the 10 rabbits, one ferret inoculated by the subcutaneous method, and one cat infected by feeding. These authors also give a preliminary report of 23 naturally infected cats. Most of these had cervical buboes and were probably infected by feeding. There were also cases of axillary and inguinal buboes and one popliteal, and the authors express the opinion that these animals were infected by fleas; two succumbed to primary pneumonic plague.

McCoy[3] working with a strain of plague originally isolated from a ground squirrel obtained the following results: Two dogs and two pigeons inoculated subcutaneously were negative; one cat inoculated intraperitoneally, four inoculated subcutaneously, and two out of four inoculated by scarification, died of plague.

In our experiments we employed, in one series of inoculations, a culture (squirrel 2010) which had been isolated a few days previously from a guinea-pig dead of acute plague after cutaneous inoculation from a naturally infected ground squirrel. In this experiment we used one pig, one calf, and one goat, all of which had survived inoculation with the plague-like disease[1] of rodents. Each animal was given one 72-hour agar culture subcutaneously. The pig and the calf showed merely a temporary indisposition. The goat was not made ill. Sixty days after the inoculation all of the animals were in good health. The guinea-pig and the white rat controls vaccinated with the same culture died of plague on the sixth and fourth days respectively.

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[1] *Jour. of Hyg.*, 1908, 8, p. 209.

[2] *Jour. of Hyg.*, 1910, 10, p. 196.

[3] *Pub. Health Bull. 43*, Pub. Health and Marine Hosp. Serv., Washington, D.C.

In the remainder of our work the strain employed (squirrel 881) had been carried for some months on artificial media. This culture was known to be virulent. It had been employed in other experimental work, including an investigation of the potency of commercial samples of anti-pest serum.

Five grown cats which had proven negative to the plague-like disease were vaccinated with a strong emulsion of a 48-hour agar culture fourth generation. A guinea-pig and a white rat were used as controls. The results are shown in the following table.

TABLE 1.

Animal	Day of Death	Result
Cat A..........	8th	Emaciated. Tubercle-like granules in liver and lungs. Guinea-pig inoculated cutaneously from liver of this cat died on 8th day with the lesions of plague
Cat B..........	9th	Necrotic area surrounded by gelatinous infiltration at site; caseous right inguinal and axillary buboes; large, dark spleen; characteristic bacilli in smears from bubo
Cat C..........	....	Killed on 17th day; no lesions
Cat D..........	....	Killed on 17th day; small necrotic gland in groin
Cat E..........	....	Killed on 17th day; necrotic gland in groin. Guinea-pig inoculated cutaneously from this gland died on 5th day with typical lesions of plague
Guinea-pig (control)	8th	Subacute plague
White rat (control)..........	4th	Acute plague

In another series we used one pig, one calf, one goat, and one sheep. Each animal was given a 48-hour agar culture subcutaneously. Two frogs and one pigeon were each given two loops of the growth. A guinea-pig and a white rat which served as controls were vaccinated with the same culture. The guinea-pig died on the twelfth day and the white rat on the fifth day. Both animals had the gross and the microscopical appearances of plague.

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[1] There is no reason for believing that this disease immunizes against plague.

The calf and the pig showed evidences of slight illness for a day or two following the inoculation. The other animals remained well. All were kept under observation for three months, during which time they showed no symptoms attributable to the inoculation, other than those mentioned.

To determine whether the plague bacillus persisted in a virulent state in the bodies of the large animals, guinea-pigs or white rats were inoculated subcutaneously with material aspirated from time to time from the site of inoculation of the calf, hog, sheep, and goat of this series. A large hypodermic syringe was loaded with about 1 c.c. of physiological salt solution. This was injected into the tissue at the site and immediately sucked back into the syringe, and the needle was withdrawn. The material was used to inoculate the test animals—guinea-pig or white rat as the case might be. The diagnosis of plague in the test animals was usually made upon gross and microscopical appearances. In a few doubtful cases, cultures were made. In Table 2 the figures denote that the test animals died of plague on that day after inoculation. The minus sign indicates a negative result; that is, the animal survived or died from other causes, or was killed and examined after the usual period with negative results.

TABLE 2.

ANIMALS INOCULATED WITH MATERIAL ASPIRATED ON VARIOUS DAYS.

	1st Day 24 Hrs. Guinea-Pig	2d Day White Rat	3d Day White Rat	4th Day White Rat	5th Day White Rat	7th Day Guinea-Pig	9th Day Guinea-Pig	12th Day Guinea-Pig	15th Day Guinea-Pig
Calf	3	2	9	—	—	5	5	—	—
Hog	3	5	3	3	3	8	—	*	—
Sheep	—	—	—	—	3	10	—	—	—
Goat	—	—	—	—	—	—	—	—	—

* Guinea-pig killed on the seventh day presented the usual lesions of subacute plague.

We are unable to account for the very evident discrepancies in the results of these inoculations. As a glance at the table shows, the goat is the only animal in which we failed to demonstrate the organism at some time in the aspirated material.

To determine the presence of antibodies in the serum of the goat, the calf, the pig, and the sheep of this series, blood was drawn from the animals on the thirty-seventh day after inoculation.

The clot was allowed to separate, and 5 c.c. of the serum was used to inject white rats subcutaneously 24 hours after the bleeding. For controls, serum was used from defibrinated blood obtained from the slaughter-house from a cow, a hog, and a sheep. No normal goat serum was available. The animals were then vaccinated with a two-day old culture of *B. pestis* of squirrel origin (No. 2010). The results are shown in Table 3.

TABLE 3.

Weight of White Rat, Grams	Serum from	Day of Death	Lesions	Remarks
65	Goat	....	None	Killed 11th day
120	Hog	....	"	Killed 11th day
240	Calf	10th	Subacute plague	
195	Sheep	8th	" "	
65	Control, no serum	4th	Acute plague	
145	Control, hog serum	3d	" "	
280	Control, calf serum	5th	" "	
230	Control, sheep serum	3d	Lungs consolidated	Cause of death not determined, probably not plague

To secure further evidence as to the protective power of the sera, white rats were given subcutaneously varying doses of the serum of the goat and of the calf of the above series. One control received a dose of normal calf serum, the other controls received no serum. These sera had been preserved for 12 days on ice with the addition of 1 per cent trikresol. The rats were then vaccinated with the bubo of a guinea-pig dead on the fourth day after inoculation from a naturally infected plague squirrel. The results are shown in Table 4.

TABLE 4.

Source of Serum	Dose	Weight of Rat, Grams	Day of Death	Lesions	Remarks
Goat	3 c.c.	90	6th	Acute plague	
Goat	2.5 c.c.	90	....	None	Killed 10th day
Goat	1 c.c.	90	....	"	Killed 10th day
Calf	3 c.c.	85	....	Caseous bubo	Killed 10th day
Calf	2.5 c.c.	95	....	Caseous foci in spleen	Killed 10th day
Calf	2.5 c.c.	135	5th	Acute plague	......
Calf	1 c.c.	110	4th	" "	......
Calf	1 c.c.	105	6th	" "	......
Calf	0.1 c.c.	100	3d	" "	......
Calf	0.1 c.c.	140	4th	" "	......
Control calf serum	2.5 c.c.	140	6th	" "	......
Control calf serum	none	130	3d	" "	......
Control calf serum	"	130	4th	" "	......
Control calf serum	"	135	4th	" "	......

The controls were all larger than the test animals and would have been expected to live longer than the latter, but as the table shows, they all succumbed to acute plague, while several of the smaller (protected) animals survived. Small doses of the calf serum exerted no protective influence.

An experiment was performed for the purpose of ascertaining whether normal calf serum plus 1 per cent trikresol had any influence on the course of plague in vaccinated white rats. The results showed that the serum-trikresol mixture was without protective properties.

We believe that these experiments indicate that the serum of the calf and that of the goat exerted decided protective action against virulent plague bacilli. Apparently the sheep and the hog serum also acted in this manner, but as only one test animal was used in each case no definite statement can be made.

SUMMARY.

Although there are a few discordant reports, the general experience with animals other than rodents and anthropoids seems to be that local and temporary constitutional effects are observed in a considerable number of cases after feeding or after subcutaneous inoculation with cultures of *B. pestis* or with plague tissues, but that fatal infections are extremely rare. The cat is an exception to this rule, and exhibits a considerable degree of susceptibility. The results of our experiments have been quite in harmony with the above generalization.

B. pestis was demonstrated at the site of inoculation after several days in the case of the calf, hog, and sheep, but not in the case of the goat.

Adequate doses of the serum of the goat and the calf inoculated with a culture of *B. pestis* certainly protected rats against plague infection. In the case of the hog and the sheep some protection was probably exerted.